Aus der Universitäts-Kinderklinik Basel
Vorsteher: Prof. Dr. A. HOTTINGER
Arbeit unter der Leitung von Frau Dr. A. BÜRGIN und Dr. M. JUST

Diagnostic Value of the Complement Fixation Reaction for Epidemic Parotitis

INAUGURALDISSERTATION

zur Erlangung der Doktorwürde
der gesamten Heilkunde der Medizinischen Fakultät der Universität Basel

vorgelegt von

JOSEPH HARTMAN
von New York (USA)

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auf Antrag von Herrn Professor Dr. A. HOTTINGER

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Introduction

The question as to the validity of the Complement fixation reaction for mumps is of general interest. The general applicability of the test renders it useful in clinically positive cases, i.e. with parotitis, as well as in clinically questionable cases, for example, serous Meningitis. The validity of serological determination of a mumps infection has already been established [1]. The question whether etiologically unclear meningitis cases may also be diagnosed through the mumps complement fixation reaction is discussed here.

Various possibilities for a laboratory diagnosis of mumps exist. These consist of the following:

1. Virus isolation.
2. The neutralization test, respectively,
   the haemagglutination-inhibition test.
3. The skin test.
4. The complement fixation reaction.

ad 1. The virus is isolated and cultivated from the saliva and cerebrospinal fluid during the first days of the disease [4, 5, 6, 7, 8]. The virus cultivation occurs either in the allantois membrane of embryonated eggs or in tissue cultures. Fluids of eggs or tissue culture medium infected with mumps virus cause haemagglutination [2, 3]. Since other viruses also cause haemagglutination (e.g. influenza virus), the reaction is not specific.
Therefore, a specific inhibition of the haemagglutination through the use of specific immune serum is undertaken. This system of identification has no great practical significance since: (a) virus isolation is practicable only in the first days of disease, and (b) it is relatively complicated.

ad 2. A known mumps virus will cause haemagglutination, as described above. The patient's serum is therefore tested for antibodies which will specifically inhibit this haemagglutination. In this system, the content of the patient's serum on antibodies is the unknown factor, whereas in 1. the virus identification is of importance.

ad 3. The technique is as follows:
Antigen is injected intradermally; the presence of antibodies in the patient's serum causes a hypersensitivity reaction. The skin reaction is read 2 days following antigene inoculation.
The skin test is not useful for the diagnosis of mumps since the hypersensitivity reaction is only noted in the convalescent period, 3–4 weeks after onset.

Complement Fixation Reactions

A complement is a heat-labile component present in most fresh animal serum. The complement fixation reaction is composed of two steps:

step 1. involves antigen and antibody (one is known, the other unknown) plus a fixed amount of pre-titrated complement. A combination of specific antigen plus antibody "fixes" the added complement.

step 2. is a test for the presence of free complement. This part of the reaction involves red blood cells plus a specific hemolysine. If the complement is "fixed" by the antigen-antibody complex there will be none available for hemolysis of the sensitized red blood cells. If, however, the complement is not fixed, there are two possible reasons for this: (a) antigen and antibody are not specific for one another, (b) either antigen or antibody are missing. If such is the case, free complement will attach itself to the sensitized red blood cells and cause lysis.

Thus a positive complement fixation reaction yields no hemolysis, whereas a negative complement fixation reaction yields hemolysis.

The mumps virus possesses two serologically distinct complement-fixing antigens, i.e. S- and V-antigens [9]. The V-antigens are closely associated with the virus, whereas the S-antigen (soluble) is considerably smaller and is found in infected tissues. Antibodies against the S-antigen appear earlier in infection than anti-V-bodies. The anti-V-bodies persist longer than the anti-S-bodies.

High levels of both antibodies indicate a recent mumps infection. Negligible anti-V titers indicate a latent or apparent infection long since overcome.

The Children's Hospital of Basel utilizes only the S-antigene in its complement fixation reaction for mumps.

Within a week following mumps infection, the building of complement-binding antibodies occurs. A quadruple increase in titer is sufficient for a mumps diagnosis as has been elicited by earlier workers [10, 11]. Also, a single titer greater than 1:64 has been accepted as sufficient for a positive diagnosis of mumps [10]. These norms are in use in the Children's Hospital of Basel and are therefore referred to as standards upon which attention will be concentrated in this discussion.

Low titers may be present in persons not having manifested a mumps infection, in which case a latent infection with the mumps virus is to be assumed.

Mumps meningitis may occur without the presence of parotitis [12]. However, the diagnosis in these cases is unclear without the presence of a positive complement fixation reaction for mumps [12].

Analysis Procedure

I. 66 cases with epidemic parotitis were taken from the records of the Children's Hospital of Basel from the years 1955–1961 inclusively.
The results of the complement fixation reaction using the S-antigen (soluble) were examined with consideration being given to the following factors:

A. a. In how many cases was the CFR negative?
How soon after the disease manifestation was the serum examined?

b. How many cases yielded low titers (less than 1:40)?
When were these sera examined?

c. How many cases yielded titers of 1:40?
When were these sera examined?

d. How many cases yielded high titers?
When were these Sera examined?

B.a. In how many cases was the serum examined more than once?

b. In how many of the cases under B.a. did the serum-titers quadruple?
How many days elapsed between the two serum removals?

II. A study was made of meningitis cases of unknown etiology.
A group of 47 cases taken from the records of the Children's Hospital of Basel in 1961 was examined under the following stipulations:

a. Only cases yielding negative results following extensive viral isolation studies were acceptable.

b. Cases used in this study should show no high titers for other specific viral diseases; e.g. polio, APC, etc.

Of interest was the following:

How many of these etiologically unclear serous meningitis cases were serologically diagnosed as mumps? On what basis was the diagnosis made?

Results

I. *Epidemic parotitis cases*

The complement fixation reaction for mumps was done in 66 cases of clinically diagnosed mumps. The cases are divided into those with a single serum examination (Table I), and those with a double serum examination (Table II).

The results of the cases with a single serum examination were as follows:

A total of 4 cases yielded *negative* titers. In none of these cases was the serum examined more than 8 days after disease manifestation. This is an indication that a serum removal for the complement fixation reaction should be performed no earlier than 8 days after the disease manifests itself. Only in such a case does the antibody-titer of the patient's serum rise to appreciable levels.

Ten cases were found with *low* titers, i.e. less than 1:40. Of these 10 cases, 8 were titer-readings where the serum was removed before the eight days elapsed. Again the antibody-titers did not have adequate time to reach appreciable levels.

There were 5 cases with titers of 1:40, which is considered the *lower* limits of a positive titer. Two of these 5 cases had serums examined after 8 days.

Sixteen cases showed *high* titers, i.e. titers of 1:80 or more. In 13 of these 16 cases the sera were removed after an 8-day period.

The results of the cases with a double serum examination were as follows (Table II):

In neither of the 2 cases yielding *negative* titers was the serum examined more than 8 days after disease manifestation.

Four cases were found with repeated *low* titers, i.e. less than 1:40. Three of these 4 cases were titer-readings with serum removal before the 8 days elapsed.

Three cases had titers of 1:40. One of these 3 cases had sera reexamined after 8 days.

High titers were found in a total of 19 cases. In 17 of these 19 cases, the serum re-examination was performed after an 8-day period had elapsed.

Etiologically unclear cases of serous meningitis

The following are the results of a survey of the cases showing meningitis, in the absence of either parotitis or virus isolation, with the complement fixation reaction for mumps:

In the year 1961, the Children's Hospital of Basel had a total of 46 cases of serous meningitis. *Seventeen* of these cases were serologically diagnosed as mumps. Of these 17 cases, 9 cases had a titer of *1:80 or more.* Another 5 cases were diagnosed on the basis of a *quadruple increase* in Titer. The three remaining cases had titers of *1:40* whereby a polio-CFR was not done. The diagnosis in these three cases must therefore remain questionable.

Table I

Synopsis of clinically diagnosed mumps cases with *single* serum removal (35 cases) (with emphasis upon time of single serum removal and consequent titers)

Days elapsed since disease manifestation	CFR results			
	Negative	Low titers	Titer 1:40	High titer
Less than 8	4	8	3	3
More than 8	0	2	2	13
Total (35 cases) . . .	4	10	5	16

table I I

Synopsis of clinically diagnosed mumps cases with *double* serum removals (31 cases) (with emphasis upon time elapsed between the two serum examinations and consequent titers)

Days elapsed between serum removals	CFR results			
	Negative	Low titers	Titer 1:40	High titer
Less than 8	2	3	2	2
More than 8	0	1	1	17
Total (31 cases)	2	4	3	19

Discussion

Mumps does not always present with the classical symptomatology ascribed to it. Diagnostic difficulties are therefore bound to occur when, for example, meningitis alone is the presenting symptom.

It is therefore important that use is made of laboratory diagnostic procedures. The complement fixation reaction is the least complicated not withstanding its reliability.

A characteristic increase in specific antibodies for mumps results in a positive complement fixation reaction. Although high titers are diagnostic for mumps, early serum removal results in titers which are deceptively low. It is therefore imperative

that that serum examination should not be undertaken before the eighth day following disease manifestation.

Of the cases with single serum removal (Table I), all of the four cases yielding negative titers had the serum examined before the 8-day interval. It may be inferred that the antibody-titer of the patient's serum had inadequate time to rise to appreciable levels.

Eight out of the 10 cases with *low* titers had the sera examined before the 8 days elapsed. Again, the antibody titers did not have adequate time to rise to appreciable levels.

Thirteen of the sixteen cases with high titers were examined after the 8-day interval, whereby a sufficient amount of time elapsed to result in positive titers.

The same conclusions may be reached upon observation of the results of the cases with double serum removals (Table II). The dependency of titer results on time elapsing between the two serum removals is noteworthy.

Of the 46 cases of serous meningitis of unclear etiology, 17 were serologically diagnosed as mumps, through the complement fixation reaction. More positive results could have been expected had more time elapsed. Thus, the complement fixation reaction is a useful method for detecting mumps in the absence of clinical mumps symptoms.

Conclusion

The complement fixation reaction is a useful tool in the diagnosis of mumps without clinical symptoms of parotitis as evidenced from cases taken from the Children's Hospital of Basel. Material gathered indicates that an 8-day interval should elapse before the serum is examined by the complement fixation reaction for mumps.

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